23. The method according to claim 22, wherein said discrete sites are wells, and said first and second microspheres are randomly distributed in said wells.

24. The method according to claim 10, wherein said substrate comprises discrete sites and said microspheres are randomly distributed on said sites.

25. The method according to claim 10, wherein said discrete sites are wells, and said microspheres are randomly distributed in said wells.

26. The method according to claim 1, 10, 22, 23, 24 or 25, wherein said substrate is a fiber optic bundle.

27. The method according to claim 1, 10, 22, 23, 24 or 25, wherein said substrate is selected from the group consisting of glass and plastic.

28. The kit according to claim 18, wherein discrete sites are wells.

29. The kit according to claim 18 or 28, wherein said substrate is a fiber optic bundle.

30. The kit according to claim 18 or 28, wherein said substrate is selected from the group consisting of glass and plastic. - -.

REMARKS

Claims 1-30 are pending. Claims 1-6, 10, 12 and 18 are amended. Claims 22-30 are new. The claims are amended for clarity and for proper antecedent basis. In addition, support for the amendment of claim 1 is found throughout the specification as filed, for example in Figure 1, p. 26, lines 16-24, and p. 24, line 34. Support for the

amendment of claim 2 is found at p. 21, line 31. Claims 3-6 are amended for proper antecedent basis. Support for the amendment of claims 10 and 18 is found at p. 24, line 34. Claim 12 is amended for clarity. Support for new claims 22 and 24 is found throughout the specification as filed, for example at p. 22, lines 31-32. Support for new claims 23, 25 and 28 is found throughout the specification as filed, for example Figure 1 and p. 24, lines 26-30. Support for new claims 26, 27, 29 and 30 is found at p. 24, lines 1-8. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made.**" For the Examiner's convenience a clean copy of the currently pending claims is appended hereto as Appendix A.

RESPONSE TO DETAILED ACTION

Priority

The Office Action indicates that the provisional application No. 60/160,027 has nothing to do with the claimed subject matter. Applicants respectfully draw the Examiner's attention to the Preliminary Amendment filed October 24, 2000 in which the claim of priority is amended to recite 60/160,927. Support for the amendment was provided in the executed Declarations filed October 24, 2000. Applicants respectfully request that the Examiner acknowledge the appropriate claim of priority and grant the present application the benefit of the 60/160,927.

Specification

The Office Action notes that the disclosure is objected to because of certain informalities. The Office Action further notes that correction is required.

A. The Office Action notes that "...clonal nucleic acids..." is unclear. However, Applicants draw the Examiner's attention to p. 21, lines 10-20 which describe clonal nucleic acids. As described herein, clonal nucleic acids are nucleic acids that are

cloned or are derived from clones.

The Office Action also indicates that IBL/DBL pairs is unclear. However, Applicants note that IBL/DBL pairs is clearly defined at p. 30, lines 23-24 and p. 31, lines 13-16.

B. The Office Action indicates that the sentence at p. 25, line 30, starting "Chemically modified sites" is unclear. However, Applicants submit that the sentence, while long, is merely a non-limiting example of various types of "chemically modified sites". No change has yet been made because it is not clear what correction the Examiner is requesting.

C. Again, the Office Action notes that IBL/DBL is unclear. For the reasons described above Applicants submit that the abbreviations are clear.

D. The Examiner notes that there are no definitions for the symbols used in Equations 1-5. Applicants submit, however, that the equations are common statistical equations that are well known to the skilled artisan. As such, definitions of the symbols are not necessary. If, however, the Examiner desires that definitions be included in the application, Applicants will amend the specification accordingly.

E. The Examiner notes that PPi is used throughout the specification and should be corrected to PP_i. However, Applicants respectfully draw the Examiner's attention to p. 2, line 14 that defines the abbreviation for pyrophosphate as "PPi". Applicants respectfully remind the Examiner that Applicants are their own lexicographers. They can define in the claims what they regard as their invention essentially in whatever terms they choose so long as the terms are not used in ways that are contrary to accepted meanings in the art. MPEP 2173.01. Applicants respectfully submit that the substitution of a normal position "i" in place of a subscript "$_i$" is not contrary to the art recognized abbreviation for pyrophosphate. As such, Applicants have not amended the specification to enact this change.

Drawings

The Office Action indicates that the drawings are objected to because they do not include reference character "50" in Figure 1C. Also, the Office Action indicates that the drawings are objected to because they include reference character "100" in Figure 1C, but it is not described in the specification.

In response, Applicants have amended the specification to recite that the "capture extender probe...**[50] 100**".... Applicants submit that the substitution of "100" for "50" in the specification finds support in Figure 1C and does not add new matter. Applicants respectfully request entry of the amendment.

Claim Objections

The Office Action indicates that claims 1, 6, 7, 13, 14 and 19 are objected to for the use of PPi instead of PP_i. Again, Applicants note that Applicants are their own lexicographers. They can define in the claims what they regard as their invention essentially in whatever terms they choose so long as the terms are not used in ways that are contrary to accepted meanings in the art. MPEP 2173.01. Accordingly, Applicants respectfully draw the Examiner's attention to p. 2, line 14 that defines the abbreviation for pyrophosphate as "PPi". As such, Applicants respectfully request the Examiner to withdraw this objection.

Double Patenting

The Office Action indicates that claims 10-11 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-2 of copending Application 09/425,633.

Applicants respectfully request the Examiner to hold this rejection in abeyance until there is an indication of otherwise allowable subject matter. Applicants will consider filing a Terminal Disclaimer if necessary.

Response to Rejection under 35 U.S.C. §112

Claims 6 and 12 are rejected under 35 U.S.C. §112, second paragraph as being indefinite for lack of proper antecedent basis. In response, Applicants have amended the claims to correct the antecedent basis. Applicants respectfully request the Examiner to withdraw the rejection.

Response to Rejection under 35 U.S.C. §102

Claims 1-3 and 6-9 are rejected under 35 U.S.C. §102(b) as being anticipated by WO 98/13523 (Nyren). Applicants note that there is now a U.S. issued patent corresponding to this PCT application, namely 6,210,891. Applicants respectfully traverse the rejection.

As the Examiner is aware, anticipation under 35 U.S.C. § 102 requires that "[f]or a prior art reference to anticipate in terms of 35 U.S.C. § 102, every element of the claimed invention must be identically shown in a single reference." In re Bond, 15 USPQ2d 1566, 1567 (Fed. Cir. 1990).

Accordingly, Applicants note that claim 1 is directed a method of sequencing first and second target nucleic acids wherein the hybridization complexes are immobilized to microspheres that are randomly distributed on a surface of a substrate.

In contrast, Nyren fails to teach sequencing first and second target nucleic acids immobilized to microspheres that are randomly distributed on a surface of a substrate. While Nyren teaches pyrosequencing of immobilized DNA, there is no mention of first and second microspheres to which first and second target nucleic acids are attached. Accordingly, Applicants submit that Nyren fails to teach each limitation of claims 1-3 and 6-9. As such, Applicants respectfully request the Examiner to withdraw this rejection.

Claims 10 and 12 are rejected under 35 U.S.C. §102(e) as being anticipated by

U.S.P.N. 6,051,380 (Sosnowski). Applicants respectfully traverse the rejection.

As noted above, for a prior art reference to anticipate a claimed invention, each element of the claimed invention must be identically shown in a single reference.

Sosnowski teaches a microelectronic device for nucleic acid assays including sequencing. There is no mention of the nucleic acids or microspheres being randomly distributed on a surface of the substrate. That is, while Sosnowski teaches nanocrystals (see Example 8), these are used as labels that are bound to DNA probes. That is, the fluorescent nanoparticles serve as a label for detection of the DNA probe. There is no teaching in Sosnowski of methods that include using a bead-bound DNA molecule in a sequencing reaction. Thus, while Sosnowski teaches sequencing, it does not teach sequencing using beads.

Claim 10 is drawn to a method of sequencing a target nucleic acid wherein the hybridization complex is attached to a microsphere randomly distributed on a surface of the substrate.

Claim 12 is directed to a method of sequencing wherein the capture probe attached to the microsphere is a sequencing primer.

In contrast, Sosnowski fails to teach methods of nucleic acid sequencing wherein the target sequence and capture probe are attached to a microsphere. Moreover, Sosnowski fails to teach that the microspheres are randomly distributed on a surface of the substrate. Finally, Sosnowski fails to teach that the capture probe attached to the microsphere is a sequencing primer. Accordingly, Applicants submit that Sosnowski fails to anticipate claims 10 and 12. Applicants respectfully request the Examiner to withdraw this rejection.

Response to Rejection under 35 U.S.C. §103

Claims 4 and 5 are rejected under 35 U.S.C. §103 as being unpatentable over Nyren et al as applied to claim 1 above and further in view of U.S.P.N. 6,083,763

(Balch).

As described above, Nyren teaches pyrosequencing of immobilized nucleic acids. Nyren fails to teach sequencing of a first and a second target nucleic acid wherein the hybridization complexes are immobilized on a microsphere that is randomly distributed on a surface. As the Examiner indicated, Nyren "do not teach hybridization complexes comprising target sequence, sequencing primer, an adapter probe and a capture probe...".

Balch teaches a high throughput method and apparatus for analyzing molecular targets in a sample. Balch mentions sequencing of nucleic acids. However, Balch fails to teach a method sequencing of first and second target nucleic acids wherein hybridization complexes are immobilized to first and second microspheres randomly distributed on a surface of a substrate. The Examiner indicates that Balch teaches that the "target probes (adapter probes) are designed to be complementary to both the capture probes and the target nucleic acids".

Basically, the position asserted in the Office Action is that it would have been obvious for one of skill in the art to combine the capture probes of Balch for the formation of hybridization complexes of Nyren. Applicants respectfully traverse the rejection.

Applicants note that there are three requirements to establish a prima facie case of obviousness. These include that "there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations." (MPEP § 2143).

Applicants respectfully submit that neither reference, taken alone or in combination, provides the motivation to practice the invention as claimed in claims 4

and 5. The Examiner suggests that the motivation to combine the references would have been that capture probes and adapter probes allowed greater flexibility in performing the hybridization reactions and screening of a large number of targets. However, Applicants note that the Examiner has failed to point to the teachings in either of the references that would have motivated the skilled artisan to combine the teachings of the references. While the Examiner has noted components of each reference, Applicants submit that the prior art makes no suggestion to modify these components to reach the present invention. The mere fact that a reference can be modified does not render the resultant modification obvious unless the prior art also provides the motivation to modify the reference to arrive at the claimed invention. *In re Mills*, 16 USPQ2d 1430 (Fed. Cir. 1990); MPEP § 2143.01.

Moreover, Applicants submit that Nyren actually teaches that when arrays of nucleic acids are used, i.e. on a microfabricated chip, they are an "ordered set of samples" that are immobilized in a 2-dimensional format. As such, Nyren teaches away from random distribution of microspheres on a surface of a substrate. As the Examiner is aware, a rejection of obviousness cannot stand based on a reference of a proposed combination of references which leads one of ordinary skill in the art away from the claimed invention. Dow Chemical Co. v. American Cyanamid Co., 2 USPQ2d 1350 (Fed. Cir. 1987). To this end, Applicants submit that a rejection under 103 cannot stand based on Nyren.

Finally, Applicants submit that the combination of the references fails to teach the claimed limitations. As noted above, the cited references do not teach the random distribution of microspheres on a surface of a substrate. Accordingly, Applicants submit that a prima facie case of obviousness has not been made. Applicants respectfully request the Examiner to withdraw the rejection.

Claims 13-16 are rejected under 35 U.S.C. §103 as being unpatentable over

Sosnowski et al applied to claim 10 above and further in view of Nyren et al.

Nyren and Sosnowski are described above. Basically, the position set forth in the Office Action is that it would have been obvious to one of ordinary skill in the art to use the pyrosequencing method of Nyren with the array of Sosnowski. Applicants respectfully traverse the rejection.

As noted above, there are three requirements to set forth a prima facie case of obviousness. These include that there is motivation found in the prior art to combine the references, the combination must provide the skilled artisan with a reasonable expectation of success in practicing the claimed invention, and the prior art references must teach or suggest all of the claim elements.

The Examiner suggests that motivation is provided in that "multiple pyrosequencing detecting reactions were carried out at the same time on a solid support array." However, Applicants note that Nyren also teaches an ordered array of nucleic acids. As such, Applicants are unclear what features of the invention are added by combining Nyren with Sosnowski.

Moreover, the Examiner states that the skilled artisan would have a reasonable expectation of success in practicing the pyrosequencing method of Nyren with the array of Sosnowski. However, Applicants note that both Sosnowski and Nyren teach ordered arrays, while the claims are directed to methods wherein microspheres are randomly distributed on a surface of a substrate. That is, as noted at p. 15, lines 1-5 of Nyren, the samples are distributed over a surface, for example a microfabricated chip, and thereby an ordered set of samples may be immobilized in a 2-dimensional format" Moreover, in Sosnowski, it is noted throughout the specification that the array is an ordered array. For example, at col. 7, lines 23-24 it is noted that the system allows for "synthesis of different oligonucleotides or peptides at specific microlocations. In addition, at col. 7, lines 35 -40 it is noted that '[a]ll microlocations can be addressed with their specific binding entities". As such, in neither Nyren nor Sosnowski is there a

teaching or suggestion to practice the inventions with an array that includes microspheres randomly distributed on a surface of a substrate. As such, Applicants submit that the skilled artisan would not have a reasonable expectation of success in practicing the invention as claimed.

Finally, as noted above, the combination of the references fails to teach or suggest all of the claim limitations. That is, there is no teaching or suggestion in either of the references to practice the inventions with an array that includes microspheres randomly distributed on a surface of a substrate. As such, Applicants submit that the Examiner has failed to set forth a prima facie case of obviousness. Applicants respectfully request the Examiner to withdraw the rejection.

Claim 11 is rejected under 35 U.S.C. §103 as being unpatentable over Sosnowski et al applied to claim 10 above and further in view of Balch.

The references are described above. Basically, the position set forth in the Office Action is that it would have been obvious to use the capture probes and adapter probes of Balch for the formation of hybridization complexes of Sosnowski. Applicants respectfully traverse the rejection.

The Examiner suggests that the skilled artisan would have been motivated to combine the references because the capture probes and adapter probes allowed greater flexibility in performing the hybridization reactions and screening of a large number of targets at the same time. However, Applicants note that the Examiner has failed to point to the teachings in either of the references that would have motivated the skilled artisan to combine the teachings of the references. While the Examiner has noted components of each reference, Applicants submit that the prior art makes no suggestion to modify these components to reach the present invention. The mere fact that a reference can be modified does not render the resultant modification obvious unless the prior art also provides the motivation to modify the reference to arrive at the

claimed invention. *In re Mills*, 16 USPQ2d 1430 (Fed. Cir. 1990); MPEP § 2143.01.

Moreover, Applicants submit that Sosnowski actually teaches that the arrays of nucleic acids are an ordered array. That is the nucleic acids are addressed. As such, Sosnowski teaches away from random distribution of microspheres on a surface of a substrate.

Finally, Applicants submit that the combination of the references fails to teach the claimed limitations. As noted above, the cited references do not teach the random distribution of microspheres on a surface of a substrate. Accordingly, Applicants submit that a prima facie case of obviousness has not been made. Applicants respectfully request the Examiner to withdraw the rejection.

Claim 17 is rejected under 35 U.S.C. §103 as being unpatentable over Sosnowski et al applied to claim 10 above and further in view of Metzker et al (Nucleic Acids Research, vol. 22, pp. 4259-4267, 1994).

Sosnowski is described above.

Metzker teaches the use of blocked dTNPs in polymerization reactions. Basically the position set forth in the Office Action is that it would have been obvious for one of ordinary skill in the art to have used the base identification method of Metzker in the array method of Sosnowski. Applicants respectfully traverse the rejection.

While the Examiner suggests that the skilled artisan would have been motivated to combine the references because the method of Metzker was performed on a solid support and used nucleotide analogs which were spectroscopically deprotected and stable during the polymerization process, Applicants submit that the Examiner has failed to point to any specific teaching in the references that suggest the motivation. That is, Applicants respectfully remind the Examiner that evidence of a motivation to combine references requires "actual evidence: That is, the showing must be clear and particular .... Broad conclusory statements regarding the teaching of multiple

references, standing alone, are not 'evidence.'" In re Dembiczak, 50, USPQ2d 1614, 1617 (CAFC, 1999). Accordingly, absent some indication of actual evidence, Applicants respectfully submit that the skilled artisan would not have been motivated to combine the references.

Moreover, Applicants submit that Sosnowski actually teaches that the arrays of nucleic acids are an ordered array. That is the nucleic acids are addressed. As such, Sosnowski teaches away from random distribution of microspheres on a surface of a substrate. As noted above, a rejection of obviousness cannot stand based on a reference of a proposed combination of references which leads one of ordinary skill in the art away from the claimed invention. Dow Chemical Co. v. American Cyanamid Co., *supra*. Accordingly, Applicants submit that a rejection under 103 cannot stand based on Sosnowski.

Finally, Applicants submit that the combination of the references fails to teach the claimed limitations. As noted above, the cited references do not teach the random distribution of microspheres on a surface of a substrate. Accordingly, Applicants submit that a prima facie case of obviousness has not been made. Applicants respectfully request the Examiner to withdraw the rejection.

Claims 18-21 are rejected under 35 U.S.C. §103 as being unpatentable over Nyren, Metzker and Sosnowski.

The references are described above.

Basically the position set forth in the Office Action is that is would have been obvious for one of skill in the art to have added the microspheres of Sosnowski with the labeled dNTPs of Metzker to kits of Nyren. Applicants respectfully traverse the rejection.

The Examiner suggests that motivation for the combination is found in that kits were conventional in the field of molecular biology and provided the benefits of

convenience and cost-effectiveness for practitioners. However, Applicants submit that the Examiner has provided no evidence of such motivation found in the prior art at the time of the present invention. Moreover, as noted above, Sosnowski and Nyren both ordered arrays; that is they teach away from arrays that include a population of microspheres randomly distributed on the surface of a substrate. Again Applicants note that a rejection of obviousness cannot stand based on a reference of a proposed combination of references which leads one of ordinary skill in the art away from the claimed invention. Dow Chemical Co. v. American Cyanamid Co., *supra*. Accordingly, Applicants submit that a rejection under 103 cannot stand based on Sosnowski or Nyren.

Finally, the combination of the references fails to teach or suggest each limitation of the claims. Accordingly, Applicants submit that the Examiner has failed to set forth a prima facie case of obviousness. Applicants respectfully request the Examiner to withdraw this rejection.

CONCLUSION

For the reasons described above, Applicants submit that the claims are now in condition for allowance. Please direct any calls in connection with this application to the undersigned at (415) 781-1989.

Respectfully submitted,

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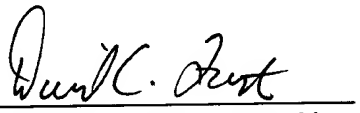

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Version with markings to show changes made

IN THE SPECIFICATION

Paragraph beginning at p. 4, line 21, has been rewritten as follows:

- -Figures 1A, 1B, 1C and 1D depict several configurations for attachment of the target sequences to the arrays of the invention. Bead arrays are depicted, although as outlined herein, any number of additional arrays may be used. Figure 1A depicts a substrate **5** with a capture probe **20** attached via an optional attachment linker **15** to an associated microsphere **10**. Target sequence **25** comprises target positions **30, 31, 32,** and **33** with a sequencing primer **40** hybridized adjacently to these positions. There may be any number of sets of target positions ($n \geq 1$). Figure 1B depicts the use of the capture probe **20** as the sequencing primer. Figure 1C depicts the use of a capture extender probe (sometimes referred to herein as an "adapter probe") **[50] 100** that has a first domain that hybridizes to the capture probe **20** and a second portion that hybridizes to the target sequence **25**. Figure 1D shows the direct attachment of the target sequence **25** to the bead **10**. - -.

In the Claims:

Claims 1-6, 10, 12 and 18 have been amended as follows:

1. (Amended) A method of sequencing [a plurality of] first and second target nucleic acids each comprising a first domain and [a] an adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:
a) providing [a plurality of] first and second hybridization complexes [each] comprising [a] first and second target sequences, respectively and [a] first and second sequencing primers, respectively, that hybridize[s] to the first domain of said first and second target sequences, respectively, said first and second hybridization complexes attached to first and second microspheres, respectively, randomly distributed on a surface of a substrate;
b) extending [each of] said first and second primers by the addition of a first nucleotide to the first detection position using a first enzyme to form [an] first and second extended primers, respectively; and
c) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said first and second primers, respectively.

2. (Amended) A method according to claim 1 wherein at least said first hybridization complex[es are] is covalently attached to said first microsphere[s distributed on said surface].

3. (Amended) A method according to claim 1 wherein at least said first sequencing primer[s are] is attached to said first microsphere [surface].

4. (Amended) A method according to claim 1 wherein [each of] said first and second hybridization complexes comprise[s] said first and second target sequences, respectively, said first and second sequencing primers, respectively, and [a] first and second capture probes, respectively, covalently attached to said first and second microspheres, respectively [surface].

5. (Amended) A method according to claim 1 wherein [each of] said first and second hybridization complexes comprise[s] said first and second target sequences, respectively, said first and second sequencing primers, [an] a first and second adapter probe, respectively, and [a] first and second capture probes, respectively, covalently attached to said first and second microspheres [surface].

6. (Amended) A method according to claim 1 further comprising:
d) extending said first and second extended primers by the addition of a second nucleotide to the second detection position using said first enzyme; and
e) detecting the release of pyrophosphate (PPi) to determine the type of said [first] second nucleotide added onto said first and second primers, respectively.

10. (Amended) A method of sequencing a target nucleic acid comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:
a) providing a hybridization complex comprising said target sequence and a capture probe covalently attached to a microsphere randomly distributed on a surface of a substrate; and
b) determining the identity of a plurality of bases at said target positions.

12. (Amended) A method according to claim 10 wherein said [sequencing primer is said] capture probe is a sequencing primer.

18. (Amended) A kit for nucleic acid sequencing comprising:
a) a composition comprising:
i) a substrate with a surface comprising discrete sites; and
ii) a population of microspheres randomly distributed on said sites;
wherein said microspheres comprise capture probes;
b) an extension enzyme; and
c) dNTPs.

The following new claims have been added:

- -22. The method according to claim 1, wherein said substrate comprises discrete sites and said first and second microspheres are randomly distributed on said sites.

23. The method according to claim 22, wherein said discrete sites are wells, and said first and second microspheres are randomly distributed in said wells.

24. The method according to claim 10, wherein said substrate comprises discrete sites and said microspheres are randomly distributed on said sites.

25. The method according to claim 10, wherein said discrete sites are wells, and said microspheres are randomly distributed in said wells.

26. The method according to claim 1, 10, 22, 23, 24 or 25, wherein said substrate is a fiber optic bundle.

27. The method according to claim 1, 10, 22, 23, 24 or 25, wherein said substrate is selected from the group consisting of glass and plastic.

28. The kit according to claim 18, wherein discrete sites are wells.

29. The kit according to claim 18 or 28, wherein said substrate is a fiber optic bundle.

30. The kit according to claim 18 or 28, wherein said substrate is selected from the group consisting of glass and plastic. - -.

Appendix A
Pending Claims

1. (Amended) A method of sequencing first and second target nucleic acids each comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:
a) providing first and second hybridization complexes comprising first and second target sequences, respectively and first and second sequencing primers, respectively, that hybridize to the first domain of said first and second target sequences, respectively, said first and second hybridization complexes attached to first and second microspheres, respectively, randomly distributed on a surface of a substrate;
b) extending said first and second primers by the addition of a first nucleotide to the first detection position using a first enzyme to form first and second extended primers, respectively; and
c) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said first and second primers, respectively.

2. (Amended) A method according to claim 1 wherein at least said first hybridization complex is covalently attached to said first microsphere.

3. (Amended) A method according to claim 1 wherein at least said first sequencing primer is attached to said first microsphere.

4. (Amended) A method according to claim 1 wherein said first and second hybridization complexes comprise said first and second target sequences, respectively, said first and second sequencing primers, respectively, and first and second capture probes, respectively, covalently attached to said first and second microspheres, respectively.

5. (Amended) A method according to claim 1 wherein said first and second hybridization complexes comprise said first and second target sequences, respectively, said first and second sequencing primers, a first and second adapter probe, respectively, and first and second capture probes, respectively, covalently attached to said first and second microspheres.

6. (Amended) A method according to claim 1 further comprising:
d) extending said first and second extended primers by the addition of a second nucleotide to the second detection position using said first enzyme; and
e) detecting the release of pyrophosphate (PPi) to determine the type of said second nucleotide added onto said first and second primers, respectively.

7. The method according to claim 1 wherein said PPi is detected by a method comprising:
a) contacting said PPi with a second enzyme that converts said PPi into ATP; and
b) detecting said ATP using a third enzyme.

8. A method according to claim 7 wherein said second enzyme is sulfurylase.

9. A method according to claim 7 wherein said third enzyme is luciferase.

10. (Amended) A method of sequencing a target nucleic acid comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:
a) providing a hybridization complex comprising said target sequence and a capture probe covalently attached to a microsphere randomly distributed on a surface of a substrate; and
b) determining the identity of a plurality of bases at said target positions.

11. A method according to claim 10 wherein said hybridization complex comprises said capture probe, an adapter probe, and said target sequence.

12. (Amended) A method according to claim 10 wherein said capture probe is a sequencing primer.

13. A method according to claim 10 wherein said determining comprises:
a) providing a sequencing primer hybridized to said second domain;
b) extending said primer by the addition of a first nucleotide to the first detection position using a first enzyme to form an extended primer;
c) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primer;
d) extending said primer by the addition of a second nucleotide to the second detection position using said enzyme; and
e) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primer.

14. The method according to claim 13 wherein said PPi is detected by a method comprising:
a) contacting said PPi with a second enzyme that converts said PPi into ATP; and
b) detecting said ATP using a third enzyme.

15. A method according to claim 14 wherein said second enzyme is sulfurylase.

16. A method according to claim 14 wherein said third enzyme is luciferase.

17. A method according to claim 10 wherein said determining comprises:
a) providing a sequencing primer hybridized to said second domain;
b) extending said primer by the addition of a first protected nucleotide using a first enzyme to form an extended primer;
c) determining the identification of said first protected nucleotide;
d) removing the protection group;
e) adding a second protected nucleotide using said enzyme; and
f) determining the identification of said second protected nucleotide.

18. (Amended) A kit for nucleic acid sequencing comprising:
a) a composition comprising:
i) a substrate with a surface comprising discrete sites; and
ii) a population of microspheres randomly distributed on said sites;
wherein said microspheres comprise capture probes;
b) an extension enzyme; and
c) dNTPs.

19. A kit according to claim 18 further comprising:
d) a second enzyme for the conversion of pyrophosphate (PPi) to ATP; and
e) a third enzyme for the detection of ATP.

20. A kit according to claim 18 wherein said dNTPs are labeled.

21. A kit according to claim 20 wherein each dNTP comprises a different label.

Please add the following new claims:

- -22. The method according to claim 1, wherein said substrate comprises discrete sites and said first and second microspheres are randomly distributed on said sites.

23. The method according to claim 22, wherein said discrete sites are wells, and said first and second microspheres are randomly distributed in said wells.

24. The method according to claim 10, wherein said substrate comprises discrete sites and said microspheres are randomly distributed on said sites.

25. The method according to claim 10, wherein said discrete sites are wells, and said

microspheres are randomly distributed in said wells.

26. The method according to claim 1, 10, 22, 23, 24 or 25, wherein said substrate is a fiber optic bundle.

27. The method according to claim 1, 10, 22, 23, 24 or 25, wherein said substrate is selected from the group consisting of glass and plastic.

28. The kit according to claim 18, wherein discrete sites are wells.

29. The kit according to claim 18 or 28, wherein said substrate is a fiber optic bundle.

30. The kit according to claim 18 or 28, wherein said substrate is selected from the group consisting of glass and plastic. - -.